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In vitro ixodicidal action of a concentrate based on plant oils on rhipicephalus microplus engorged female ticks

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ABSTRACT

R. microplus is an obligate hematophagous ectoparasite of cattle that causes billions of dollars in losses in the global livestock economy and thus we evaluate the efficacy of a concentrate, using vegetable oils on the mortality of engorged female *R. microplus* ticks. For each group, 15 ticks were used and assigned to four treatments: T1-T4 and a control group (CG), that were not exposed to the extract at all. The ticks were immersed in each treatment for a period of 15 minutes. Upon completion, the specimens were removed from the container and the excess of product was removed using blotting paper. To determine mortality, the ticks were placed in Petri dishes and left to rest inside an extraction hood at 31.6 °C and 56% relative humidity and were observed every 24 hours for seven days. In the T1, mortality was 73% at 48 h post-treatment, while in the T2 - T4 groups, mortality was 100% at 24 h. The result shows that mixed vegetable oil extracts are a viable alternative for the control of *R. microplus*. We suggest further studies on its efficacy in vitro and in vivo using lower concentrations.

Contribution/Originality: This study contributes to the knowledge about the use of plant mixed oil extract on the mortality of cattle ticks as an alternative to chemical control methods in a subtropical region of Mexico.

1. INTRODUCTION

Globally, ticks (Acari: Ixodidae), obligate blood-sucking ectoparasites (Uspensky, 2017) are known to cause the most economic losses in livestock production (Domínguez, Agatón, & Cruz, 2016) either directly through irritation resulting from their bites, which may generate discomfort leading to stress, that may interfere with the animal's feeding, resulting in indirect losses like decrease in meat production, milk production and delayed growth (Rodriguez-Vivas, Jonsson, & Bhushan, 2018). Ticks can also act as vectors of pathogens of public health importance (Kasaija, Estrada-Peña, Contreras, Kirunda, & De La Fuente, 2021).

The main method of tick control is the application of chemical acaricides. The downside to this control method includes; acaricide resistance brought about by frequent and indiscriminate use of these products, and secondary effects on the environment, which in fact influences the presence of chemical residues in foods of animal origin

(Ostfeld, Price, Hornbostel, Benjamin, & Keesing, 2006; Vudriko et al., 2018). To mitigate this, alternative control methods are being explored, especially the use of natural plant products for the control of arthropods of veterinary economic importance (Showler & Saelao, 2022). Luns et al. (2021) and Wanzala, Hassanali, Mukabana, and Takken (2014) have reported some essential oils to be acaricidal or repellent against ticks. These compounds are also safe either by exposure or through consumption for humans, animals, and the environment (Selles et al., 2021). This study thus sought to evaluate the acaricide effect of a mixture of five essential oils using the tick immersion test on engorged female *R. microplus* ticks.

2. MATERIALS AND METHODS

2.1. Tick Collection Site

Maldonado community (16°29´1.0" N and -98°36´28.0" W) in Cuajinicuilapa, Guerrero, México is a town with small livestock production units, dedicated to the breeding, milking and sale of bovines. Tick collection was carried out in August 2021.

2.2. Experimental Setup and Design

The experimental part was carried out in the Multidisciplinary Laboratory of the Faculty of Veterinary Medicine and Zootechnics No. 2 of the Autonomous University of Guerrero in the municipality of Cuajinicuilapa. The municipality is located between 16°28'34" N and -98°25'46" W, at an altitude of 46 masl. The climate is warm and sub-humid with an annual rainfall of 1,100 - 1,300 mm, temperature range of 24 - 26°C. and an average humidity of 97% (National Institute of Statistics and Geography - INEGI, 2010).

2.2.1. Ticks

Only engorged adult female *R. microplus* ticks were used throughout this study. These ticks were selected and classified according to their physical appearance, size, and mobility. All ticks that showed no signs of mobility or appeared physically weak were discarded. Ticks with very wide disparities with respect to size, as with those that showed visible physical damage were exempted from the experiment. Sample size was calculated using references described by Marín-Quintero and Vargas-García (2017) and Rojas and Sánchez (2017). In this way, 75 engorged female *R. microplus* ticks were selected, and 15 individuals were each assigned to form a group; a control group (CG) and four treatments: T1-T4 groups. Controlled conditions for maintenance include, temperature set at 31.6 °C, humidity at 56 %, and 12-h photoperiod.

2.2.2. Oil Extracts (Ultracic®)

Ultracic® - Agronaturalia is a natural agricultural adjuvant composed of a mixture of selected natural oils with insecticide effects. As declared in the technical data sheet, the product contains the following oils: almond, cedar, cinnamon, corn, geranium, jojoba, lemongrass, and soybean.

2.2.3. Treatment Preparation

For this purpose, 2.5, 5.0, 7.5, and 10.0 mL of Ultracic® - Agronaturalia (Product) were diluted in 7.5, 5.0, 2.5, and 0.0 mL, of distilled water dilutions to obtain a final volume of 10 mL. Quantitation for each treatment was achieved using sterile plastic syringes (Plastipak® - 10 mL) and placed in plastic labeled disposable cups (Plastic World®). Each treatment was grouped as follows: CG = 100% distilled water (Dw), T1 = 25% product (P) / 75% Dw, T2 = 50% P / 50% Dw, T3= 75% P / 25 % ml Dw, and T4 = 100% P.

2.2.4. Experimental Model

The ticks assigned to each group were placed in a permeable container that was then submerged in the diluted solutions for 15 minutes. The time was set using an electronic timer and the ticks were extracted from the solution and the container upon the completion of the set time. Blotting paper was employed for the removal of the excess solution. Each tick was returned to their Petri dish. The observation of the specimens was carried out at a 24-hour interval as described by Bravo, Coronado, and Henríquez (2008).

2.2.5. Mortality Measurement

To observe the effect of treatment, the ticks were placed on sheets marked with red circles and observed for mobility for a period of 10 minutes (Giannelli, Dantas-Torres, & Otranto, 2012). Failure to show movement of its legs, or motility in general, was considered as dead (Rojas & Sánchez, 2017). The mortality of ticks were measured according to the methodology of Marín-Quintero and Vargas-García (2017).

2.3. Data Analysis

Data obtained during the study were subjected to descriptive statistics and expressed as percentages.

3. RESULTS

During the study, the vegetable oil-based concentrate had an ixodicidal effect on engorged *R. microplus* female ticks. Ticks of the CG maintained alive up to the day 6th. On the T1, it is possible to observe a mortality of 73% at 72 h post-treatment, while in the remaining treatments (T2-T4) the mortality of the specimens was 100% at 24 h. In the Figure 1, the number of dead specimens is presented with respect to the hours of treatment.

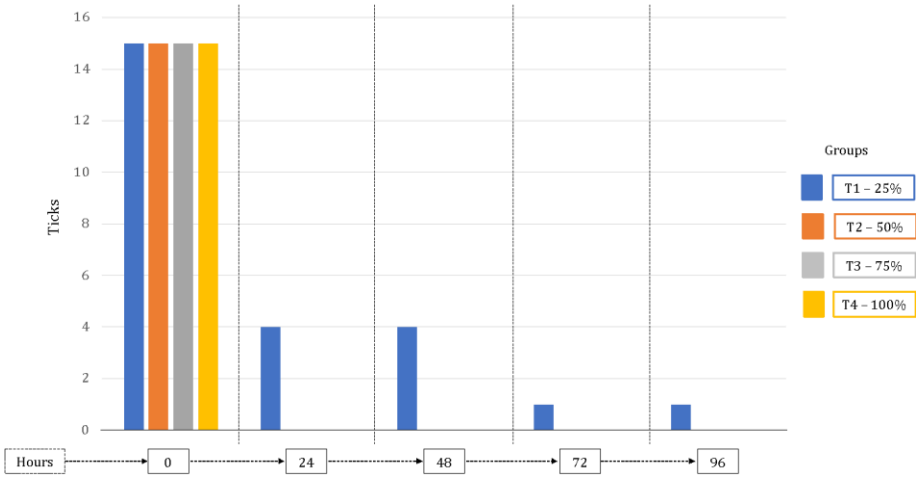

Figure 1. Tick mortality with respect to exposure time.

4. DISCUSSION

The lifespan of CG ticks extended to more than day 6. Our observation is in line with the reported by Castelblanco Sepúlveda, Sanabria Rodríguez, Cruz Carrillo, and Rodríguez Molano (2013) who mention that after immersion in distilled water, the mortality of ticks must not to exceed 10%. In other hand, in the treated ticks, Gil et al. (2013) mentions that some substances present in plants act dissolving the lipids of the cuticle of the exoskeleton; while others generate paralysis and finally the death of the arthropods (Rodríguez, Rodríguez, & Cruz, 2010). Martinez-Lopez (2008) mentions that the mortality of *Sitophilus zeamais* at 24 hours after the treatment of the corn seed with almond oil obtained a mortality of 38%. Merino et al. (2012) argues that treatments with almond oils, under laboratory conditions have a mortality rate of 98% - 100% of the adult population of *S. zeamais*. Although so far there are no studies that support the use of almond oil as an ixodicide in ticks, this would be the

first evidence of the use of this product. It is recommended to evaluate almond extract on its own to determine its ixodicidal effect. There are no studies on the use of cedarwood oil with an ixodicidal effect on ticks. However, Naseem, Faheem-Malik, and Munir (2016) mentions that cedar oil has properties such as repellent of flies, moths, and mosquitoes. While Flor-Weiler, Behle, Eller, Muturi, and Rooney (2022) demonstrated that cedarwood oil has a repellency of 50% on *Amblyomma americanum*, *Dermacentor variabilis*, *Ixodes scapularis*, and *Rhipicephalus sanguineus* ticks. Boito et al. (2018) reported the insecticidal effect of *Cinnamomum zeylanicum* on *Musca domestica* with mortality ranges from 70 – 89% after 60 and 240 minutes at 5% of concentration. For its part, Álvarez, Loaiza, Bonilla, and Barrios (2008) mentions that cinnamon extract at its maximum concentration (pure), has 100% mortality in ticks, while in 1:1 dilution of the extract in water it decreased to 2.18% mortality. Until now there are no studies of the corn extract that prove the ixodicidal effect on ticks; however, Cuevas-Salgado, Castañeda-Templos, and Romero-Nápoles (2015) used corn oil in adult females to control the prickly pear mealybug *Dactylopius opuntiae*, having a mortality of 77%. In this way, Merino et al. (2012) reports the effect of corn oil on *S. zeamais* with a mortality of 100%. Cressman and Dawsey (1942) mentions that the components of corn oil have insecticidal effects by means of a mechanism of excitation of the nervous system in insects. However, there are no studies that prove the use of corn oil in ectoparasites, and this would be the first investigation that contains this product. da Silva (2017) reports that using geranium oil, the bean weevil (*Acanthoscelides obtectus*) has a mortality of 80% at 24 h post-exposition. The geranium oil was proved in combination with other oils, as repellent of the tick *Ixodes ricinus* (Jaenson, Garboui, & Pålsson, 2006). However, its use as an acaricide has not been reported so far. No studies have been found that support the use of jojoba oil as an ixodicide on ticks. Jojoba oil is suggested as a safe product with a potential for use as a bioinsecticide (Al-Obaidi et al., 2017; Ismail, Wahba, Hassan, & Haker, 2021). In his study, Abdel-Razik and Mahmoud (2017) mentions that jojoba oil has toxic effects, alter the feeding and egg-laying behavior of insects. As well, Ismail and Shaker (2014) reports a 76% of mortality using jojoba oil against the Immature stages of *Spodoptera littoralis*. It is recommended to evaluate the jojoba extract by itself to determine its ixodicide effect. Soybean oil has been proved as mosquito repellent with poor results. In their studies, Fradin and Day (2002) and Campbell and Gries (2010) reports that in people using different concentrations of this oil has not a good repellent effect. In contrast, Chávez et al. (2010) report that, with soybean oil against *S. zeamaiz*, observed a mortality of 50% of at 144 h, 94% at 168 h, and 100% at 192 h. Elsewhere it is recommended the use of soybean oil as repellent; however, it use is more employed in mosquito control. For this reason, the use of this oil as a tool for control of ticks needs to be investigated.

5. CONCLUSION

All treatments caused 100% mortality over the ticks subjected to the experiment, even in the lowest concentration solution. Mixed vegetable oil extracts are a viable alternative for the control of *R. microplus*. It is necessary to carry out studies on its efficacy in dilutions of less than 25%, and to prove the efficacy of the product *in vivo*, considering that the use of ecological products to avoid chemical products.

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Institutional Review Board Statement: The Ethical Committee of the Universidad Autónoma de Guerrero, México has granted approval for this study on 1 June 2021 (Ref. No. 2-010).
Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.
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